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Enzyme-Linked Immunosorbent Assay-IgG Antibody Avidity Test for Single Sample Serologic Evaluation of Measles Vaccines

Vanda Akico Ueda Fick de Souza,[1]* Cláudio Sérgio Pannuti,[1] Laura Masami Sumita,[1] and Heitor Franco de Andrade, Jr.[2]

[1]*Department of Infectious Diseases and Laboratory of Virology, Instituto de Medicina Tropical de São Paulo, Faculty of Medicine, University of São Paulo, São Paulo, Brazil*

[2]*Department of Pathology and Laboratory of Protozoology, Instituto de Medicina Tropical de São Paulo, Faculty of Medicine, University of São Paulo, São Paulo, Brazil*

A measles-specific enzyme-linked immunosorbent assay (ELISA)-IgG avidity test for serologic evaluation of the efficacy of measles vaccines with only one blood sample was evaluated after vaccination with three measles vaccine strains. Avidity indices were determined by the urea elution technique in samples presenting antibody titers ≥100 mIU/ml. All 127 sera collected 2–8 weeks after primary vaccination with Biken-CAM70 measles vaccine had low avidity indices (LAI, when ≤29%) with a time-dependent increase in avidity. In samples collected 6–10 weeks after vaccination with Edmonston-Zagreb, LAI were also observed in all 31 sera tested (mean = 15%) and in 233/242 (96.3%) filter paper samples from primary vaccination with Schwarz vaccine (mean = 14%). There was no difference in the mean avidity among the three groups of primary vaccinees, although the Schwarz group had higher antibody titers. In contrast, only 1/36 (2.8%) serum samples from children who were seropositive at the time of measles vaccination had LAI (mean = 56%), despite the fact that they were collected early (2–5 weeks after vaccination). Of 90 serum samples from children vaccinated in the past with two doses and of 42 cord blood serum samples, none had LAI.

It is concluded that this test is a good tool for evaluating serologically the efficacy of a single dose schedule of measles vaccine. With only one postvaccination sample, the test can discriminate nonresponders (antibody titers below 100 mIU/ml), primary responders (antibody titers ≥100 mIU/ml with LAI), and those previously immunized (antibody titers ≥100 mIU/ml with high avidity indices). The seroconversion rate can be calculated after excluding the latter. ***J. Med. Virol. 52:275–279, 1997.***

KEY WORDS: measles; vaccine; immunoglobulin G; avidity

INTRODUCTION

The efficacy of measles vaccines is often evaluated by the assessment of the immunological response that is measured by the rates of seroconversion as well as the geometric mean titer (GMT) of the post-vaccination samples. According to the recommendation of the World Health Organization [1988], seroconversion is defined by a negative to positive or by a fourfold increase from preimmunization titers measured in general by three serological tests hemagglutination-inhibition (HI), plaque reduction neutralization test (PRN), and enzyme-linked immunosorbent assay (ELISA). Using either test, antibody titers of at least 100 mIU/ml in the postvaccination samples, determined in relation to the international standard anti-measles serum, are considered as protective levels.

The determination of the prevaccination serologic status is an important step in order to exclude from vaccine trials those children previously stimulated by measles antigens, since incorrect information about previous immunization is common in developing countries [Sabin et al., 1983; Pannuti et al., 1987]. The main disadvantage of this procedure is the requirement for two blood samples, one obtained prior to vaccination and the other 6–8 weeks after vaccination, which is the recommended time interval for testing antibodies after primary measles vaccination. Additionally, the sero-

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*Correspondence to: Vanda Akico Ueda Fick de Souza, Av. Dr.Eneas de Carvalho Aguiar, 470 - São Paulo - 05403-000 - Brazil. Email:vaueda@usp.br

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negativity in a prevaccination sample measured by the less sensitive tests commonly used, such as ELISA or HI, does not ensure that the vaccinees had never been stimulated before because secondary vaccine failures can occur after vaccination [Hayden, 1979; Mathias et al., 1989]. In this circumstance, a secondary response may take place after further immunization [Erdman et al., 1993; Hidaka et al., 1994].

One approach to discriminating primary from secondary response after a immunization is to assess the affinity (or avidity for complex antigens) of IgG antibodies. The IgG antibody response to an antigen is an ordered process that evolves with increasing antibody avidity, essentially by somatic mutation and selection of specific B cells [Nossal, 1992]. Thus, the low avidity IgG antibodies produced in the early stage of primary immune response are gradually replaced by high avidity antibodies. However, after restimulation, the earliest antibodies produced are already of high avidity [Eisen and Siskind, 1964; Steiner and Eisen, 1967]. Therefore, the estimation of IgG avidity in the postvaccination serum may distinguish primary from secondary response without the requirement of the prevaccination sample.

Several methods for estimating the avidity of IgG antibodies have been developed for the diagnosis of primary acute viral infections [Inouye, et al., 1984; Thomas and Morgan-Capner, 1991; Hedman et al., 1993]. The most commonly used method was introduced by Hedman and Seppala [1988]. It is based on the elution by urea of the low avidity antibodies bound to the antigen from the solid phase of the ELISA [Blackburn et al., 1991; Meurman et al., 1992; Junker and Tilley, 1994; Ward et al., 1994; Tuokko, 1995]. This approach was used to an in-house ELISA for the estimation of measles-specific IgG avidity after vaccination, determining in parallel the antibody titers with the aim of evaluating its usefulness for serologic evaluation of the efficacy of a measles vaccine schedule using only one postvaccination sample.

MATERIALS AND METHODS

Sera

The sera belonged to specific groups (groups 1–4) from several standardized programs for testing measles vaccine. These programs were implemented by the Public Health Services of São Paulo State, after approval by The Ethics Committee.

Group 1 (primary immunization) included children who were vaccinated at 9 months with measles vaccine. At the time of vaccination, they had no history of previous immunization or measles antibodies in prevaccination samples. This group included 127 serum samples collected 2–8 weeks after vaccination with the Biken-CAM70 measles vaccine (BK), 31 serum samples collected 6–10 weeks after vaccination with the Edmonston-Zagreb strain (EZ) intradermally, and 242 filter paper blood samples obtained 6–10 weeks after vaccination with the Schwarz strain (SW). Group 2 (reinfection) included 36 serum samples from children who were seropositive at the time of vaccination with the BK measles vaccine. The samples were collected 2–5 weeks after vaccination. Group 3 (past immunization) included 90 serum samples collected 5–17 months after vaccination with two doses of the BK measles vaccine (at 6 and 11 months). Finally, group 4 (transplacental antibodies) included 42 cord blood serum samples from children born to mothers aged 30–42 years.

IgG Antibody Assay

Measles antibodies were detected using an in-house ELISA. Briefly, to prepare measles antigen, Vero cells were infected with the Toyoshima strain of measles virus. After incubation at 37°C for 4–5 days, when extensive CPE was observed, the cells were scraped from the flasks and washed twice with phosphate-buffered saline (PBS) by 10-min centrifugation at 800*g*. The pellet was resuspended in PBS in a volume equivalent to a 1:50 of the original medium. After sonication, measles antigen was extracted by treatment with an equal volume of 0.2% sodium deoxycholate during 1 hour at 4°C and clarified by centrifugation. Control antigen was obtained similarly from uninfected Vero cells. Microtiter plates (Hemobag, Brazil) were coated with 50 μl of previously standardized measles virus or control antigens diluted in PBS and incubated for 2 hours at room temperature. After washing twice with PBS containing 0.1% Tween 80 (PBST), they were incubated for 1 hour at 37°C with blocking solution (5% defatted milk [Molico-Nestlé®] in PBST) and a 1:100 serum dilution was dispensed in duplicate into wells coated with either virus or control antigen. After incubation for 1 hour at 37°C, the plates were washed four times with PBST and horsereadish peroxidase-conjugated anti-human IgG (Sigma, USA) was added to each well and incubated for 40 minutes at 37°C. After four washes with PBST, chromogenic substrate (0.02% hydrogen peroxide and 0.05% o-phenylenediamine in 0.05 M Na citrate buffer, pH 5.0) was added. After 20 minutes at room temperature in the dark, the enzymatic reaction was stopped with 2.5 N H_2SO_4 and absorbance was read at 492 nm in a Titertek Multiskan Plus II apparatus. The results were expressed as the value of the control antigen OD subtracted from the virus antigen OD in each serum sample (ΔOD).

The test was standardized previously by comparison with the PRN test and presented 98.9% sensitivity, 100% specificity, and 0.92 correlation between antibody titers and ΔOD. The cut-off of the test was 0.12, calculated by the mean ΔOD plus 3 SDs of negative samples [Souza et al., 1991].

Standard serum (National Institute for Biological Standards and Control, Hertford, England) diluted to contain 20, 10, 5, 2, and 1 mIU/ml was included in each set of tests. Antibody titers of the samples, expressed in mIU/ml, were calculated by a linear regression analysis and multiplied by the dilution factor (100 or 1,000).

IgG Antibody Avidity Assay

IgG antibody avidity was tested using a wash with urea, as described elsewhere [Hedman and Rousseau, 1989]. Briefly, after incubation of diluted serum for 1 hour at 37°C, the plates were washed twice with PBST. One half of the wells were soaked in PBST containing urea and the other half with PBST without urea. After two additional washes with PBST, bound IgG was detected as described above. The avidity index (AI), expressed as a percentage, was calculated by (ΔOD with urea/ΔOD without urea) × 100. Comparison between AI for standardization of urea washing was made using an Epi-Info 6.0 software. The kappa index was calculated between tests. After definition of the homogeneity of variances between samples and groups, analysis of variance (ANOVA) tests were carried out to define significant data.

RESULTS

Determination of Urea Concentration and Elution Time

The optimum concentration of urea and the elution time were determined using 37 serum samples from the group of reinfection and past immunization (A) and 22 serum samples from the group of primary immunization (B). They were tested at the same time with 6, 7, and 8 M urea for 5 minutes and with 7 M urea for 10 minutes (Fig. 1). 6 M urea/5 minutes (groups 1A and 1B) resulted in a small loss of OD in treated samples, with only few low values of AI in group B. The 99% confidence cut-off for low antibody avidity (LAI), estimated by the average of group B sera plus 3 SDs, was high (80%) and resulted in a low kappa index of 0.543. Using the same criteria, 7 M urea/5 minutes (groups 2A and 2B) gave a cut-off of 38%, with one intercrossed sample. Washing with 7 M urea/10 minutes (groups 3A and 3B) or 8 M urea/5 minutes (groups 4A and 4B) gave similar results. Both had a kappa index of 100% when tested with a cut-off of 31% and 19%, respectively.

In this study we chose the 7 M/10-minute schedule and the cut-off of the AI was defined as 29%, calculated as above with additional 22 postvaccination samples collected 6–8 weeks after primary vaccination. Therefore, a sample was defined as having a LAI if its AI was ≤29%.

In a few instances, the 1:100 serum dilution resulted in absorbances above the limit of the ELISA reader in the wells untreated with urea. When the ΔOD was >1.8, serum samples were tested again at 1:1,000 dilution to permit a more accurate calculation of the AI.

IgG Avidity After Primary Vaccination

The results of the AI and GMT of all sera used in the study are shown in Table I. Only sera presenting antibody titers ≥100 mIU/ml were included in the analysis of avidity. After primary vaccination with the BK measles vaccine, all 127 serum samples collected between 2 and 8 weeks had LAI, with a mean AI = 8.3 ± 4.6, presenting a clear increase with time (Fig. 2). The nine serum samples collected 6–8 weeks after vaccination with this vaccine had a mean AI of 14.3 ± 3.1 and a GMT of 412 mIU/ml.

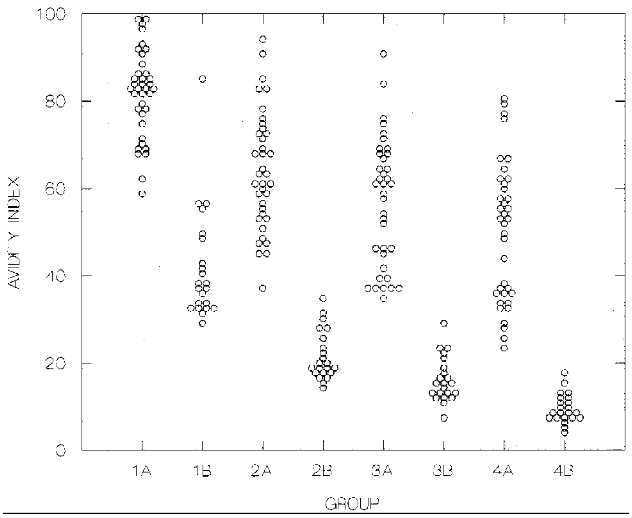

Fig. 1. Avidity indices in reinfection/past infection (A) and in primary immunization (B) with different elution time and concentration of urea. Groups: 1 (6 M urea/5 minutes), 2 (7 M urea/5 minutes), 3 (7 M urea/10 minutes), and 4 (8 M urea/5 minutes).

All 31 EZ sera collected 6–10 weeks after vaccination with the EZ vaccine could also be defined as LAI (mean AI = 15.1 ± 4.8). After vaccination with the SW vaccine, those samples were collected at the same period as for the EZ group, 233/242 (96.3%) could be defined as LAI (mean AI = 14.5 ± 7.2), despite the fact that these samples were collected and stored on filter papers.

In samples collected 6–10 weeks after vaccination, ANOVA showed no significant difference in the mean AI between subjects vaccinated with the three different strains ($P > 0.8$) nor in the proportion of sera with LAI ($P > 0.2$), but the SW vaccinees had higher GMT ($P < 0.05$) than the BK and EZ groups.

IgG Avidity After Reinfection/Past Immunization and Cord Sera

Only 1 of 36 (2.8%) vaccinated children who were seropositive at the time of measles vaccination (group 2) had LAI (mean AI = 56.2 ± 15.6) in their postvaccination samples that were collected 2–5 weeks after vaccination. Twenty-six children in this group had no history of previous immunization, but were seropositive before vaccination. The remaining 10 children who were previously seropositive had a history of immunization. This group had significantly higher GMT when compared to other groups ($P < 0.01$).

None of the 90 samples from group 3 (past immunization) that were collected 5–17 months after vaccination with two doses of measles vaccine at 6 and 11 months of age had LAI (mean AI = 66.3 ± 15.5). Forty-two cord sera also had no LAI (mean AI = 66.6 ± 17.1).

DISCUSSION

In an attempt to improve methods for serologic evaluation of the efficacy of measles vaccines, we evalu-

TABLE I. Measles IgG Avidity After Primary Vaccination, Reinfection, and in Past Infection and Cord Sera

Group	No.	No. (%) of samples with low AI	GMT (mIU/ml)	ΔOD without urea (mean)	ΔOD with urea (mean)	AI (mean ± SD)
Primary vaccination						
Biken-CAM70 (BK)	127	127 (100.0)	387	0.38–1.70 (0.77)	0.01–0.37 (0.07)	0.2–20.9 (8.3 ± 4.6)
Edmonston-Zagreb (EZ)	31	31 (100.0)	384	0.41–1.71 (0.76)	0.04–0.40 (0.12)	7.8–25.6 (15.1 ± 4.8)
Schwarz (SW)	242	233 (96.3)	907*	0.39–1.74 (0.87)	0.01–0.54 (0.13)	0.7–48.8 (14.5 ± 7.2)
Reinfection	36	1 (2.8)	10,084**	0.60–1.70 (1.28)	0.31–1.50 (0.73)	28.4–90.4 (56.2 ± 15.6)
Past immunization (Two doses of BK at 6 and 11 months)	90	0 (0.0)	1,234	0.38–1.76 (1.18)	0.14–1.65 (0.85)	33.2–94.3 (66.3 ± 15.5)
Cord sera	42	0 (0.0)	3,808	0.77–1.73 (1.27)	0.27–1.72 (0.87)	34.9–99.6 (66.6 ± 17.1)

*Significantly higher when compared to other primary vaccination groups ($P < 0.05$).
**Significantly higher when compared to other groups ($P < 0.01$).

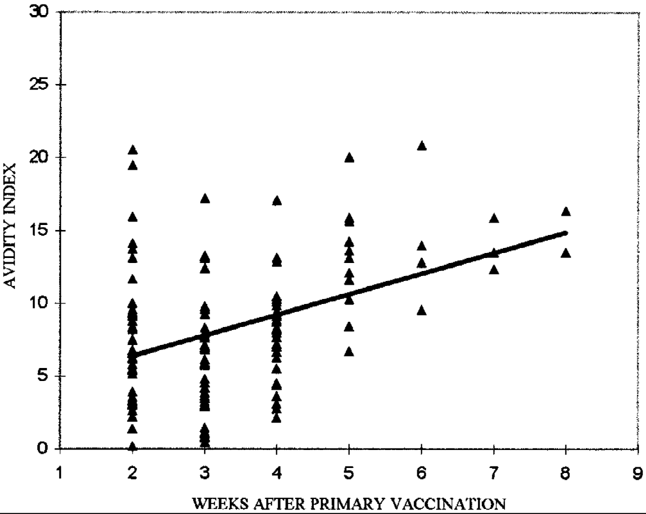

Fig. 2. AI of sera with more than 100 mIU/ml anti-measles antibody, according to the time (weeks) after primary vaccination with the BK measles vaccine.

ated an ELISA-IgG avidity assay using a single-step urea washing and a single 1:100 initial dilution to facilitate large-scale application.

Variability of results has been reported when different commercial plates and different sources of antigen or coating techniques are used when testing avidity with ELISA [Inouye et al., 1984; Thomas and Morgan-Campner, 1991]. Different concentrations of urea and elution time have also been employed for different antigens [Hedman and Rousseau, 1989; Hedman et al., 1989a; Blackburn et al., 1991]. Thus, in the present study, different schedules of urea washing were tested in an attempt to standardize the procedure for the in-house ELISA assay. Similar results were observed when 7 M urea/10 minutes with a cut-off of 31% or 8 M urea/5 minutes with a cut-off of 19% was used. However, we chose the former schedule because the cut-off was similar to that previously reported by Hedman and Rousseau [1989] in order to facilitate the comparison with other studies. We also considered this schedule technically more feasible when the test was applied to a great number of plates, because of the better control of the time of urea washing. As the standardization of urea washing was undertaken with random serum samples from the primary immunization group, we also determined the cut-off for measles IgG avidity with 7 M urea/10 minutes in additional selected sera collected 6–8 weeks after primary vaccination. This selection was based on the recommendations of the WHO [1988] for the collection of postvaccination samples during measles vaccine trials. Its value (29%), determined using a strict 99% confidence interval, was quite similar to that obtained previously.

Since an international standard anti-measles serum diluted to contain variable concentrations of antibody was included in each set of tests, the data from the ΔOD of the wells washed with PBST without urea could be used in parallel to calculate the antibody titers by a linear regression analysis. In all tests, the correlation between ΔOD and antibody titers was >0.9.

Although all samples collected 2–8 weeks after primary vaccination with the BK vaccine had LAI, a clear time-dependent increase of the AI after primary vaccination with a good parametric correlation could be observed (Fig. 2). Similar results were reported by Hedman et al. [1989b] who evaluated IgG avidity after rubella vaccination.

As delays in postvaccination sample collection are common during vaccine trials, we also evaluated the test for a time interval up to 10 weeks after vaccination. Six to ten weeks after primary vaccination, all samples from the EZ group and 233/242 (96.3%) of the SW group had LAI.

Our results show that this avidity test can be used to evaluate different vaccine strains. The analysis of the data from the three groups of vaccinees, taking into account the same range of time interval for postvaccination sample collection (6–10 weeks), showed no significant difference in the mean AI of the three groups, although the SW group had higher GMT than the EZ

and BK groups, as reported by others [Berry et al., 1992; Bolotovski et al., 1994].

Nevertheless, all but one postvaccination sera from the reinfection group had high avidity indices (mean AI = 56.2 ± 15.6) and high antibody titers (GMT = 10,084), even when collected early (2–5 weeks after vaccination), providing a clear-cut difference between primary and secondary response. This single LAI serum in the reinfection group was obtained from a 9-month-old child without a history of immunization according to his mother's information. However, the prevaccination sample also had low avidity antibodies and the postvaccination serum of this child was collected only 2 weeks after vaccination. These findings indicate that a recent infection could have occurred in this child. Incorrect maternal information about measles vaccination, which is common in developing countries [Pannuti et al., 1987; Sabin et al., 1983], or an asymptomatic infection at that time could explain this finding. A postvaccination puncture at an appropriate time (6–8 weeks) would probably result in a high avidity index and could be distinguished from primary immunization.

The test was also specific in past infection sera since none of the 90 children vaccinated with two doses of the BK measles vaccine (at 6 and 11 months), whose sera were collected 5–17 months later, had LAI. The same was observed in 42 cord blood sera.

This study demonstrates that this ELISA-IgG antibody avidity test is a good tool for serologic evaluation of a one-dose schedule of measles vaccine. With only one postvaccination sample obtained within an appropriate time interval after vaccination (6–10 weeks), the test enables definition of three groups: the nonresponders (postvaccination samples with antibody titers below 100 mIU/ml), the primary responders (antibody titers ≥100 mIU/ml with LAI), and the group of previously immunized children (antibody titers ≥100 mIU/ml with high avidity indices). Therefore, the seroconversion rate and GMT can be calculated after excluding the latter.

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